

UNIVERSITY OF IOWA STUDIES

STUDIES IN CHILD WELFARE

VOLUME I

NUMBER 5

INVESTIGATIONS IN THE ARTIFICIAL FEEDING OF CHILDREN

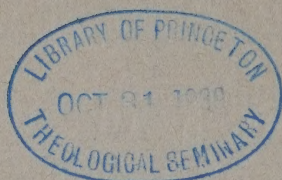
BY

AMY L. DANIELS
ALBERT H. BYFIELD
with the coöperation of
ROSEMARY LOUGHLIN

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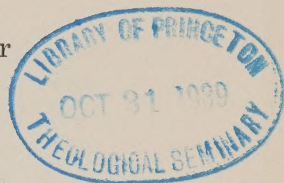


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UNIVERSITY OF IOWA STUDIES IN CHILD WELFARE

PROFESSOR BIRD T. BALDWIN, PH. D., Editor



FROM THE IOWA CHILD WELFARE RESEARCH STATION

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INVESTIGATIONS IN THE ARTIFICIAL FEEDING OF CHILDREN

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AMY L. DANIELS, PH. D.
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ROSEMARY LOUGHLIN, M. S.

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January, 1921

EDITOR'S FOREWORD

In coöperation with the Department of Pediatrics of the College of Medicine, the Division of Nutrition of the Iowa Child Welfare Research Station has begun a series of investigations on the nutrition of infants and school children. The three papers included in this series present the results of investigations regarding the effect of the addition of materials containing the so-called antineuritic vitamin to the milk mixtures of artificially fed infants. A study has also been made of the effect of heat on the nutritional value of milk.

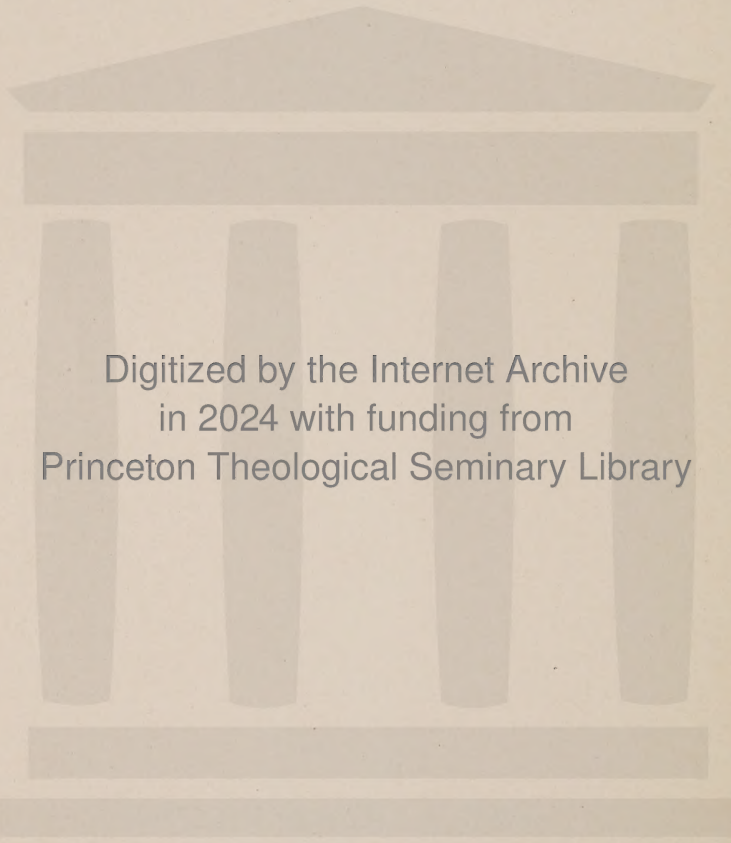
In the first paper, the effects of the growth stimulating value of antineuritic material obtained from wheat embryo is shown through consecutive observations in weight on six children ranging in age from one and one-half months to five months. A similar positive effect of the extract of vegetables is shown in the increments of weight of two children ranging in age from two and one-half months to four and one-half months. Vegetable soup used as a part diluent in milk showed like effects in the increase in weight of a child four and one-half months old. The second paper reports experiments showing that orange juice not only contains the anti-scorbutic vitamin, but its growth stimulating properties appear to be due to the latter vitamin. The third paper gives the results from a series of experiments with animals fed *heat-treated milks*. The effects on the growth curves of rats are noted when milk is brought quickly and slowly to the boiling temperature; similar comparisons are made with diets of pasteurized, evaporated, and fresh milk.

BIRD T. BALDWIN.

Office of the Director,
Iowa Child Welfare Research Station,
University of Iowa,
Iowa City, Iowa.
January, 1921.

The Rôle of the Antineuritic Vitamin in the
Artificial Feeding of Children

AMY L. DANIELS, PH.D.
AND
ALBERT H. BYFIELD, M.D.
WITH THE COOPERATION OF
ROSEMARY LOUGHLIN, M.S.
IOWA CITY, IOWA



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THE RÔLE OF THE ANTINEURITIC VITAMIN IN THE ARTIFICIAL FEEDING OF INFANTS*

AMY L. DANIELS, PH.D., AND ALBERT H. BYFIELD, M.D.

WITH THE COOPERATION OF
ROSEMARY LOUGHLIN, M.S.
IOWA CITY, IOWA

From time immemorial substitutes for human milk have been sought in the mammary secretions of other animals. The inadequacy of such substitutes has been made apparent by the distinctly higher incidence of disease and death among artificially fed babies. Early attempts were directed toward the correction of the grosser difficulties. By variously modifying and diluting the substituted milks—more particularly cow's milk—these have been rendered more acceptable to the digestion and quantitative needs of the infant. As a result, artificial feeding in the hands of the skilful worker has been fairly successful if measured in terms of disease and death. But so concerned have we been with the more obvious disturbances that little attention has been paid to the possible deficiencies of these diluted milks which may render them greatly inferior to human milk as far as their growth promoting factors are concerned, although experience has taught that the addition of other substances to the milk diet has been found to be not only desirable but necessary as early as the sixth month.

While students of nutrition have for some time been awake to the importance of the antineuritic vitamin as an essential factor in growth and physiologic well being, with the exception of cases of beriberi, there has been little consideration of a possible relationship between this growth factor and nutritional disturbances in infancy. The reason for this is possibly due to the fact that a paucity of this material produces such subtle changes that the interest of the pediatrician has not been drawn to them, especially since until very recently we have believed milk to be a valuable source of this food accessory.

That milk is comparatively low in the antineuritic vitamin has been pointed out by both Gibson and Concepcion¹ and Osborne and Mendel.² The former investigators, working with dogs and pigs

* From the Department of Nutrition, Iowa Child Welfare Research Station and the Department of Pediatrics, State University of Iowa.

1. Gibson, R. B., and Concepcion, I.: *Philippine J. Sc. B.* **11**:119 (May) 1916.

2. Osborne, T. B., and Mendel, L. B.: *J. Biol. Chem.* **34**:537 (June) 1918.

on problems pertaining to infantile beriberi, found that the addition of milk to diets consisting largely of polished rice did not protect against symptoms of polyneuritis, a disease which is known to be caused by a lack of this food accessory; while Osborne and Mendel demonstrated its low concentration through feeding experiments with rats, where milk was the sole source of the vitamin. Both groups of investigators have suggested that some of the difficulties in artificially fed babies may be due to an insufficient amount of the vitamin material. Indeed, Gibson and Concepcion believe that even in breast fed babies the diet should be extended as soon as possible. These state that "The young of healthy mothers probably come into the world with a reserve supply of the vitamin substances sufficient to tide them over nutritively until the time when under natural conditions of life they begin to eat other foodstuffs." If this is true of the breast fed baby, how much more important it is that the diet of the artificially fed baby be scrutinized with this in mind. To quote Osborne and Mendel³: "A particular case of this kind is that of infant feeding where it is customary to reinforce the supply of calories by diluting top milk and adding milk sugar. Under these circumstances the child is supplied with a food that contains a relatively smaller proportion of the water soluble vitamin than does the original cow's milk. While milk thus modified may contain sufficient vitamin as long as the food intake is normal, if for any reason the child's appetite fails, the vitamin supply is reduced and endless dietary troubles may easily result."

In a study of the weight charts of the babies in our clinic it was observed that in order to get adequate gain in weight it was necessary to give considerably more food than when breast milk is used. Whereas 100⁴ calories per kg. is considered sufficient for breast fed babies under 6 months of age, and 90 calories for those more than 6 months old, our babies were receiving from 120 to as high as 150 calories per kilogram on their "theoretical"⁵ weight. This experience apparently is borne out by other workers,⁶ although these authors do not state whether or not they have based their requirements on the theoretical or the actual weights.

3. Osborne, T. B., and Mendel, L. B.: Loc. cit.

4. Langstein, L., and Meyer, L. F.: *Säuglingsernährung und Säuglingsstoffwechsel*, 2d Ed., Wiesbaden, 1914, p. 98.

5. The following adaptation of Finkelstein's rule was used in estimating the theoretical weight: Birth weight + (600 times age in months) — 300 = weight for the first six months. Birth weight + (500 times age in months) = weight for the second six months.

6. Morse, J. L., and Talbot, F. R.: *Diseases of Nutrition and Infant Feeding*, New York, The Macmillan Co., 1915, p. 185. Hess, J. H.: *Principles and Practice of Infant Feeding*, Philadelphia, F. A. Davis Co., 1918, p. 148.

The cases considered were all normal babies many of whom had been in the clinic from the eighteenth day. The feedings consisted of milk mixtures approximating the composition of mother's milk. The dilution system of modification was used, cream and one or more of the usual carbohydrates being added, with sometimes cereal diluents. In all cases the milk preparations were either pasteurized, or boiled one minute in an open kettle. With these mixtures in such liberal quantities, eminently satisfactory gain was achieved, for it was the custom when the child failed to gain to give as much food as was necessary to obtain a normal growth curve. Scurvy was protected against by one-half ounce of orange juice given daily.

If milk is, as suggested, deficient in the antineuritic vitamin it is possible that the large caloric requirement of our babies may be explained by the fact that the additional food, beyond that necessary to meet the needs when breast milk is fed, carried enough of this essential material by adsorption to supply the needs of the child. McCollum⁷ and co-workers found in feeding experiments with rats that when the 20 per cent. of lactose used in their purified ration was replaced by an equal amount of dextrin, no growth was secured. These authors attribute their results to a lack of the antineuritic vitamin which had been introduced into the ration with the lactose. Observations, therefore, on the effect of the addition of materials known to contain the antineuritic vitamin were made. During the ward walk those children who were not gaining were selected for a study of the influence of the antineuritic vitamin, without regard to the calory value of the food intake. These babies were receiving from 104 to 126 calories per kilogram on their actual weights and from 90 to 130 calories on their theoretical weights. In each case from one to three periods of vitamin additions were made to the diets, with intervals of about ten days between each. The length of the periods during which the material containing the antineuritic vitamin was added varied from ten to twenty days.

In the first series the antineuritic material was obtained from wheat embryo, the method of extraction being the same as that used in studies pertaining to its influence on growth in animals (rats) fed purified rations. One hundred and eighty gm. of the embryo were extracted with 95 per cent. alcohol for from forty-eight to ninety-six hours. After filtering, 50 c.c. of water were added, the alcohol was distilled off, and the residue made up to 500 c.c. with distilled water. This vitamin containing mixture when tested with Fehling's solution caused a slight reduction; the addition of Lugol's solution produced a faint violet cloud. Subsequent hydrolysis yielded

7. McCollum, E. V., and Davis, M.: J. Biol. Chem. **20**:641, 1915.

a fluid which contained 2.3 per cent. of reducing sugars.⁸ Since 50 c.c. were used in each day's feeding the amount of carbohydrate added was equivalent to approximately 1 gm., not enough to affect the growth curve materially.

In a second series (Chart 6) the influence of the alcoholic extract of those vegetables—carrots, turnips and celery— which were used in preparing the vegetable soup referred to below was tested. The vegetables were comminuted, dried in a current of air and treated in the same way as the wheat embryo. The residue of the alcoholic extract of 675 gm. (fresh weight) of vegetable was dissolved in 500 c.c. of distilled water. This mixture contained less than 1 per cent. of reducing sugars. In certain instances 50 c.c. of this were added to a day's feeding, in others, 80 c.c.

DISCUSSION OF RESULTS

It will be seen from a study of the charts that when the vitamin containing extracts were added there was a similar gain in weight in all cases, in spite of the variable factor, such as age, the caloric value of the food and the somewhat different percentage composition of the mixtures fed. In the five charts (Charts 1, 2, 3, 4 and 5) showing the results of the wheat embryo additions, the ages of the children varied from 1½ to 5 months. A similar stimulation of growth took place in older children to whose diet the antineuritic vitamin was added. But since the food conditions in these were not constant, frequently including slightly variable amounts of cereals, their growth curves have not been included.

Apparently the caloric value of the food had little influence on our results, for the food ingestion per kilogram on the actual weight varied between 100 and 120 calories and between 88 and 130 calories on the theoretic weights. Furthermore, in Chart 5 it will be observed that in the same baby the caloric value of the food per unit of weight diminished with the increase in weight, while the growth stimulating influence of the added material was uniform during the periods studied. At the beginning of the investigation this baby (Dorothy G.) was receiving 120 calories on her actual weight, whereas at the beginning of the third period of vitamin addition she was receiving only 100 calories on her actual weight.

In Chart 4 we were able to demonstrate that the slight carbohydrate content of the antineuritic extracts obtained from both the wheat embryo and the vegetables was without influence. The addition of 7 gm. of dextri-maltose did not materially affect the growth curve

8. Benedict, S. R.: J. A. M. A. **57**:1193 (Oct. 7) 1911.

in this case (Jean M.) When, however, the antineuritic vitamin containing extract was added there was a stimulation of growth which apparently was otherwise not possible, for growth had become stationary on the very low food intake—88 calories per kilogram on her theoretical weight—which this baby was receiving at this period.

It is appreciated that if the daily addition of an antineuritic material must be made to the diet of the artificially fed baby some

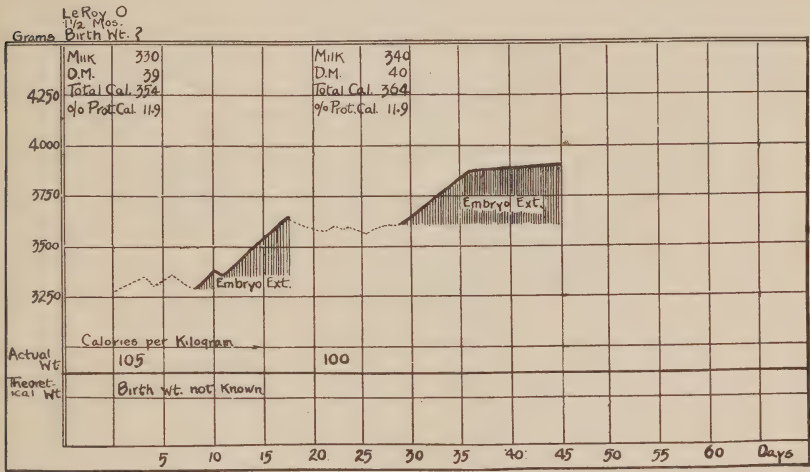


Fig. 1.—The influence of addition, withdrawal and second addition of the wheat embryo extract.

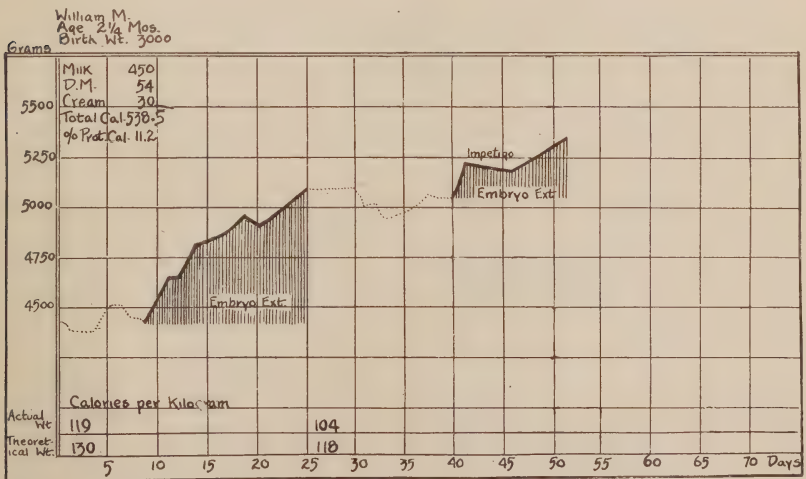


Fig. 2.—When the caloric food intake is considerably above the average, growth stimulation by the embryo extract appears to be somewhat greater. (See Chart 4.)

more easily available source than the wheat embryo extract must be found. Vegetables naturally were first thought of in this connection; and in Charts 6 and 7 are shown curves which demonstrate that the antineuritic material contained in these is a possible source when given in sufficient quantity. In the case of Harriet B. (Chart 6) a slight gain was achieved when 50 c.c. of the prepared vegetable extract were used; and a still more characteristic increase when 80 c.c.

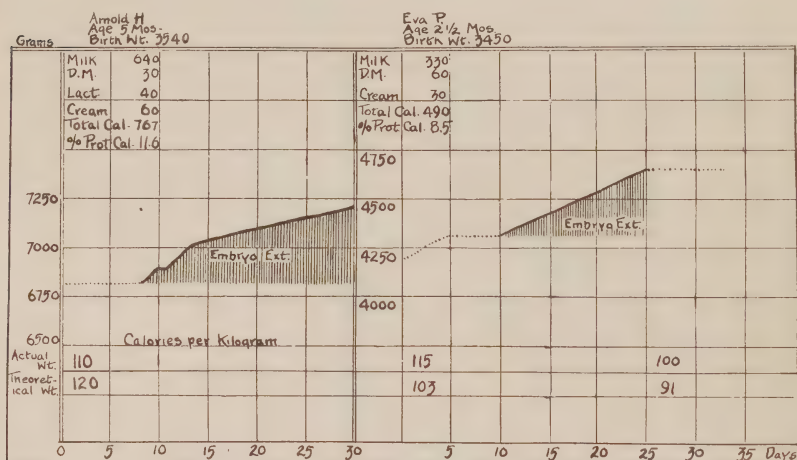


Fig. 3.—Stimulation of growth brought about by the daily addition of the wheat embryo extract following periods of practically stationary weight.

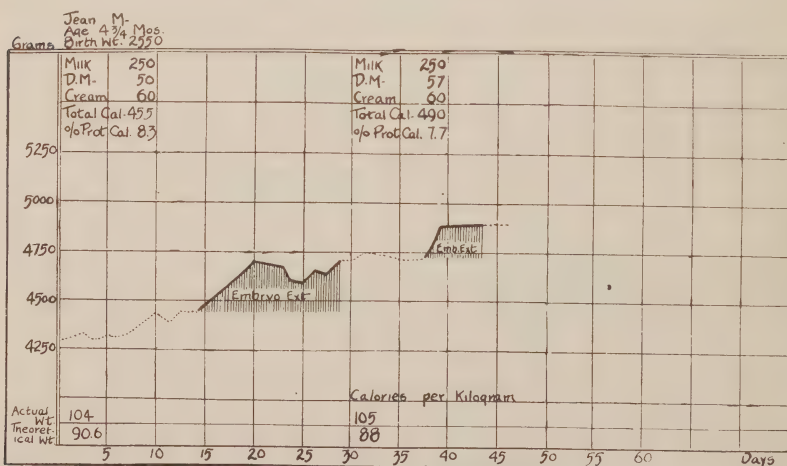


Fig. 4.—With smaller caloric intake—less than 95 per kg.—wheat embryo extract has a lesser influence in bringing about weight increases. The addition of 7 gm. of carbohydrate (28 calories) was without influence on the weight curve.

were included in each day's diet, the material being added to the bottle feedings.

Since our preparations of the antineuritic vitamin are not feasible in routine infant feeding, a special vegetable soup was substituted for the extracted material, and used as a part diluent in the milk formula. This was made of 227 gm. of turnips, 278 gm. of carrots and 170 gm. of celery. These were comminuted and cooked until

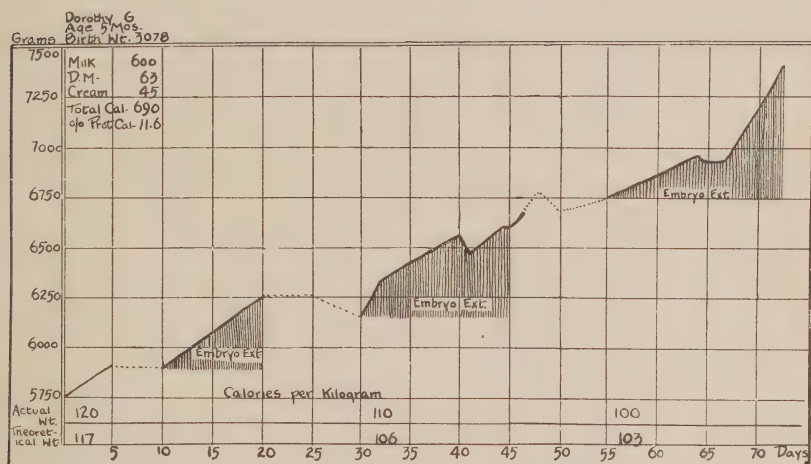


Fig. 5.—Favorable influence of wheat embryo extract addition during three successive periods.

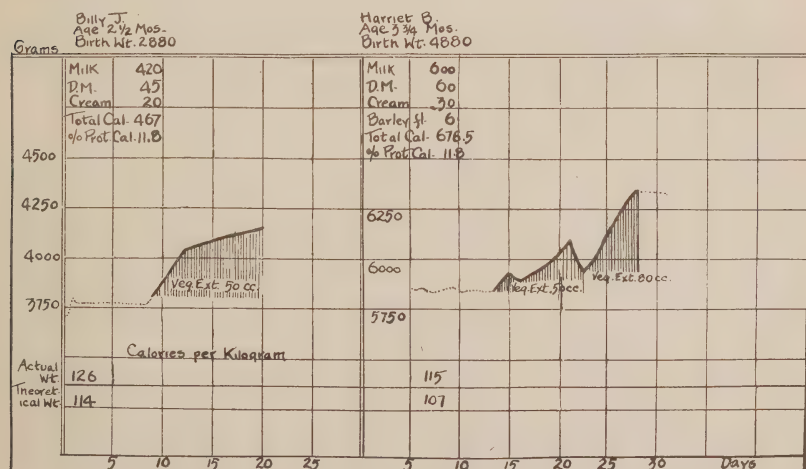


Fig. 6.—The alcoholic extract of vegetables had an influence similar to that of the wheat embryo extract. In the case of Harriet B., 80 c.c. seemed somewhat more efficient than 50 c.c.

very soft, with enough water to cover. The material was then strained, thereby removing practically all of the cellular material. From the 675 gm. of fresh vegetables there was obtained 500 c.c. of soup.

In Chart 7, 50 c.c. of this vegetable soup were found to be insufficient. When double this amount was added growth was markedly stimulated. With the use of the vegetables, it is appreciated that another factor has been introduced, namely, that of the inorganic salts. What part these salts play in the growth curve and what their effect on the child is, remains to be determined. There is no doubt, however, that at least a part of the growth stimulation has been due to the antineuritic vitamin.

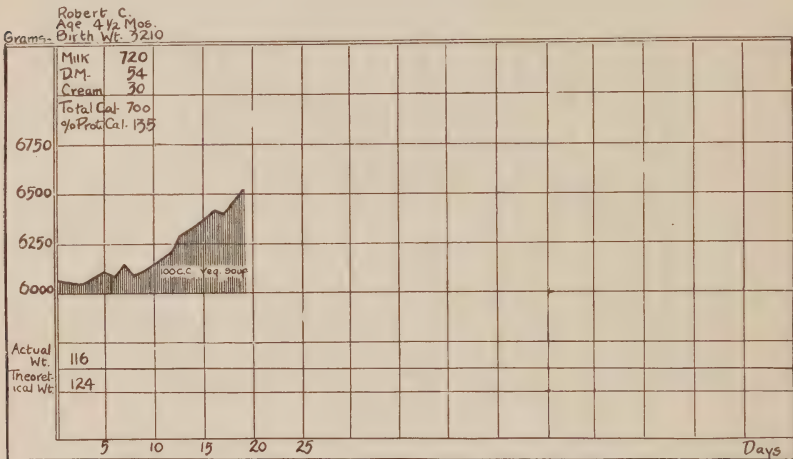


Fig. 7.—Vegetable soup used as part diluent in the milk modification also stimulated growth. During the first part of the investigation period 30 c.c. were added, whereas during the second part 100 c.c. were used.

It should be borne in mind that the infants reported here have all been normal babies and therefore their antineuritic requirements were presumably small. However, this should not affect the validity of the findings for in other cases in which this substance has been added where there was manifestly under nutrition, growth has been stimulated. Since other therapeutic measures were included in the treatment of these cases it has not seemed pertinent to include their charts in this report. Our results here are comparable apparently to those of Eddy and Roper⁹ who found that the antineuritic vitamin obtained from pancreas stimulated growth in children suffering from marasmus.

9. Eddy, W. H., and Roper, C. J.: *Am. J. Dis. Child.* **14**:189 (Sept.) 1917.

The influence of the addition of the antineuritic vitamin on the growth of older children has been indicated by Hess.¹⁰ In studies pertaining to infantile scurvy, a cereal composed of wheat middlings and farina was used. "In certain instances," he states, "improvement was immediate and striking; there was a gain in weight for the first time in many months." The effect of this material, however, was in no way comparable to that produced by the addition of orange juice. It is probable that Hess' babies were suffering from a lack of the antineuritic as well as the antiscorbutic vitamin. In a later attempt to find a substitute for orange juice as an antiscorbutic, Hess¹¹ in turn used both yeast and wheat germ preparations. While his work indicated that these apparently have no antiscorbutic value, when they were added to the diet of some older babies and children from 1½ to 2 years old, there was marked stimulation of growth. It would appear these children also had been receiving a diet furnishing too little of the antineuritic vitamin.

CONCLUSIONS

From the results of the investigation it would seem that the following conclusions are justified:

1. The addition of the antineuritic vitamin obtained from wheat embryo to the diet of babies supplied with food furnishing an adequate number of calories stimulated growth.

2. The beneficial influence of adding a specially prepared vegetable soup in sufficient quantity as part diluent in the milk modifications for infants is apparently due to the presence of the antineuritic vitamin contained therein. Both the alcoholic soluble material of the dried soup vegetables, and the water extract (soup) stimulated growth.

3. The fact that the artificially fed infant requires a larger amount of food than the breast fed infant appears to be due to the relative paucity of diluted cow's milk in the antineuritic vitamin.

4. It is probable that failure to gain in infants and young children is often the result of an insufficient amount of the antineuritic vitamin in the food. The diets of the young, we believe, should be more carefully scrutinized with this in mind.

10. Hess, A. F.: J. A. M. A. **65**:1003 (Sept. 18) 1915.

11. Hess, A. F.: Am. J. Dis. Child. **13**:98 (Jan.) 1917.

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AMERICAN MEDICAL ASSOCIATION
FIVE HUNDRED AND THIRTY-FIVE NORTH DEARBORN STREET
CHICAGO

THE ANTINEURITIC AND GROWTH STIMULATING PROPERTIES OF ORANGE JUICE *

ALBERT H. BYFIELD, M.D., AND AMY L. DANIELS, Ph.D.

WITH THE COOPERATION OF

ROSEMARY LOUGHLIN, M.S.

IOWA CITY

Orange juice has been so universally regarded as an antiscorbutic, that its other possible properties as affecting the well-being of artificially fed infants have not received much consideration. This, perhaps, is to be expected in view of the fact that the daily giving of orange juice is comparatively recent.¹ Orange juice has been widely used as a mild cathartic for infants and young children, its potency having been assumed. Recently, Gerstenberger² has pointed out that there is no experimental basis for this, and states that, on the contrary, orange juice tends to constipate. The diuretic property of orange juice has been noted by Gerstenberger,³ and again by Hess.⁴ Orange juice was the chief constituent of a fruit mixture used by Gladstone⁵ in treating certain cases of marasmus. This mixture, of which he used a surprisingly large amount—24 ounces a day—consisted of 2 parts of orange and 1 part apple juice with a small amount of water. According to the author's report, this was enjoyed by all babies, who soon became less restless and irritable, but did not gain in weight until a suitable milk modification was given. The good results were attributed to the "tonic cleansing effects on the mucous membranes" and to the "diuretic, diaphoretic and general alterative properties" of the mixture. Kohlbrugge⁶ has reported the benefits following the administration of orange juice in cholera infantum.

The therapeutic effects of the addition of orange juice to the diets of infants suffering from scurvy has been studied by Hess.⁷ Accord-

* From the Department of Pediatrics and the Child Welfare Research Station, State University of Iowa.

1. Hess, J. H.: *Principles and Practice of Infant Feeding*, Ed. 2, Philadelphia, 1919, p. 156. Hill, L. W., and Gerstley, J. R.: *Clinical Lectures on Infant Feeding*, Philadelphia, 1917, p. 160. Morse, J. L., and Talbot, F. B.: *Diseases of Nutrition and Infant Feeding*, New York, 1915, p. 233.

2. Gerstenberger, H. J., and Champion, W. M.: *Am. J. Dis. Child.* **18**:88 (Aug.) 1919.

3. Gerstenberger, H. J.: *Am. J. M. Sc.* **155**:253 (Feb.) 1918.

4. Hess, A. F.: *Am. J. Dis. Child.* **14**:337 (Nov.) 1917.

5. Gladstone, H. B.: *Practitioner*, London **97**:472 (Nov.) 1916.

6. Kohlbrugge: *Centralbl. f. Bakteriöl.* **60**:223 (Part 1) 1911.

7. Hess, A. F.: *J. A. M. A.* **75**:1003 (Sept. 18) 1915. Hess, A. F.: *Am. J. Dis. Child.* **12**:152 (Aug.) 1916.

ing to him, some of the symptoms of infantile scurvy appear to bear a close relationship to the deficiency diseases—more particularly beriberi; for besides the usual signs and hemorrhagic symptoms of scurvy, he found such others as tachycardia, dilation of the heart and failure to gain. When orange juice was given, not only did the usual scurvy symptoms disappear, but the children gained in weight and the cardiac signs became normal. The omission of the orange juice was followed by a period of stationary weight until it was again added to the diet. These gains the author attributed to the effect of the antiscorbutic material. In the reports, unfortunately, there are not sufficient data to determine the caloric value of the food given or to indicate its content of growth promoting material. Those children who continued to gain in spite of scorbutic symptoms may have been receiving more food or food supplying more of the antineuritic vitamin.⁸ Our previous work showing the influence on growth of the addition of this vitamin to the diet of babies led us to suspect that Hess' weight gains following the addition of orange juice might be owing to the presence of antineuritic material in the orange juice, rather than to the antiscorbutic material.

Hitherto, oranges, and fruits in general, although valuable antiscorbutics, have not been regarded as sources of the antineuritic vitamin. The literature contains no mention of them in the treatment of beriberi, and with the exception of the banana and the tomato, so far as we have been able to find, there have been no experiments indicating their antineuritic properties.⁹ The present state of our information on this point is suggested by the following quotation from Harden and Zilva:¹⁰ "We have so far not come across a natural product which contained both the antiscorbutic and the antineuritic vitamins in quantities suitable for investigation." In order to obtain a mixture containing both vitamins, these investigators added autolyzed yeast to orange juice to supply the antineuritic material.

Since the antineuritic value of oranges had not been determined, it seemed pertinent to study them from this standpoint, especially in respect to their influence on growth. In our clinic, a series of observations were carried out on babies under the same conditions as those reported in a previous communication.¹¹ With one exception, the diet of the infants was constant throughout the various periods, the intake per kilogram being computed both on the theoretical and actual

8. We shall use the term "antineuritic" vitamin, recognizing the fact that this may include more than one substance as suggested by Mitchell, H. H.: *J. Biol. Chem.* **11**:399 (Dec.) 1919.

9. Daniels, A. L., and Byfield, A. H.: *Am. J. Dis. Child.* **18**:546 (Dec.) 1919

10. Harden, A., and Zilva, S. S.: *Biochem. J.* **12**:93 (June) 1918.

11. See Note 9.

weights. The milk mixtures were "sterilized" by boiling one minute in an open kettle. In all cases these babies had been receiving from the first month a daily dose of 15 c.c of orange juice—the customary amount given in this clinic. From preliminary studies of the influence of orange juice on the growth of rats, it was roughly estimated that 45 c.c. of orange juice should stimulate growth. Accordingly, this amount, properly diluted, and sweetened with a few drops of a saccharin solution, was given, one half in the morning and one half in the afternoon, to those infants whose weight had remained stationary for a number of days. For some days previous, and during the observation periods, both the food and the orange juice were prepared by one of us (A. L. D.).

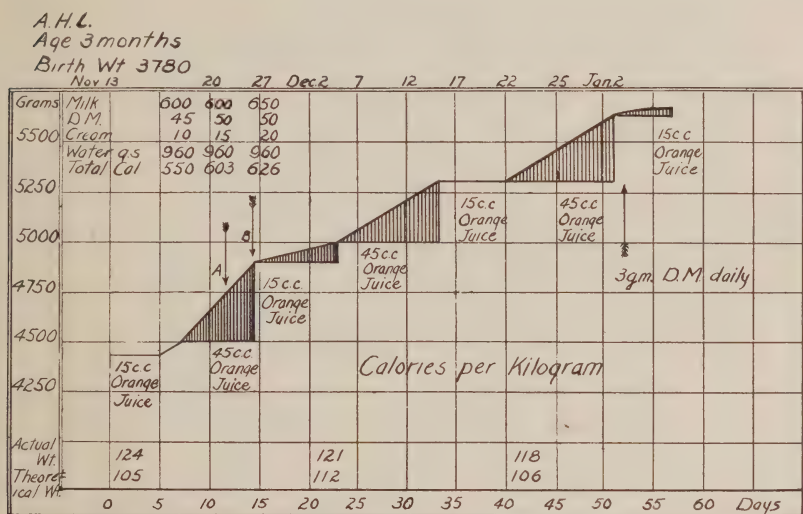


Fig. 1.—A. H. L. Three separate orange juice additions in a period of sixty days gave uniform weight increases. During Period 1, an increase in the food produced no corresponding increase in weight. When the usual quantity of orange juice (15 c.c.) was given at B, a second increase in food was made. The effect of the two food increases was distinctly less than that produced by the larger quantity of orange juice.

It will be noted that in every case when the amount of orange juice was increased from 15 c.c. to 45 c.c. per day, there was a marked stimulation of growth. When the amount of orange juice was reduced to the 15 c.c., the weights again became stationary. The longest observation was in the case of A. H. L. (Fig. 1), three separate orange juice periods being included. During period one it was necessary to increase the amount of food, owing to the fact that the baby was extremely hungry and restless. A food increase of fifty-three calories had no apparent influence on the rate of gain. The day after the orange juice

was removed, the food was again increased by twenty-three calories. This produced only a slight gain (100 gm. in ten days), and was in no way comparable to that produced by the orange juice (250 gm. in five days). The subsequent addition of orange juice during a period of nine days resulted in an increase in weight of 300 gm. A second decrease in the amount of orange juice was again followed by a stationary weight period. A third addition of the larger amount of orange juice stimulated growth as before. The weight curves of the other babies show similar results, and are comparable to those of our earlier work in which it was shown that under similar conditions growth was stimulated by the addition to the milk formula of the anti-neuritic vitamin obtained from the wheat embryo extract.

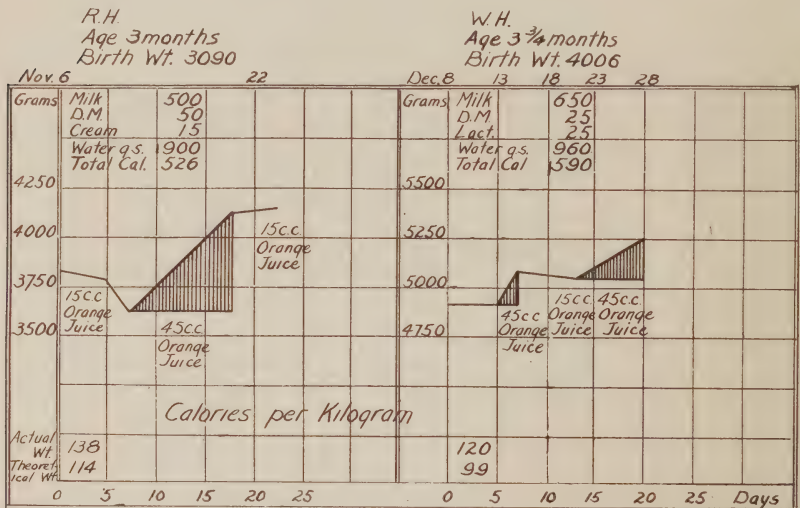


Fig. 2.—R. H. and W. H. The addition of 45 c.c. of orange juice per day produces an average daily weight gain of 40 gm. in the case of R. H.; with W. H. the immediate effect of the addition of orange juice during ten days is shown. A reduction to 15 c.c. produced a stationary weight.

Seidell,¹² and later, Harden and Zilva¹³ have shown that the anti-neuritic vitamin may be removed quantitatively from a substance containing the two vitamins by adsorption, either with Lloyd's reagent or fuller's earth, the antiscorbutic material remaining unaffected. Accordingly, in our work the expressed juice (80 c.c.) of the orange was shaken with 15 gm. of kaolin for twenty minutes and filtered. When 45 c.c. of this filtrate per day were given to the babies there was no increase in weight (Figs. 3 and 4). In the following period, however,

12. Seidell, A.: U. S. Public Health Rep. **31**:366, 1916.

13. See Note 10.

when an equal quantity of *untreated* orange juice was given, there was an immediate gain in weight. From these results it appears that the growth stimulating factor had been removed by the kaolin.

That orange juice contains a growth stimulating material is further evidenced by the fact that rats fed a purified ration, with orange juice as the sole source of the antineutritic vitamin, grew normally, although a larger amount (75 c.c. per 100 gm. of ration) was necessary to produce the same rate of gain as when our wheat embryo extract was used (25 c.c. per hundred grams of ration) (Fig. 5, Group I). This was not due to the antiscorbutic vitamin because other rats receiving orange juice which had been boiled for five minutes with an excess of a 2 per cent. solution of sodium hydroxid (to destroy the antiscorbutic

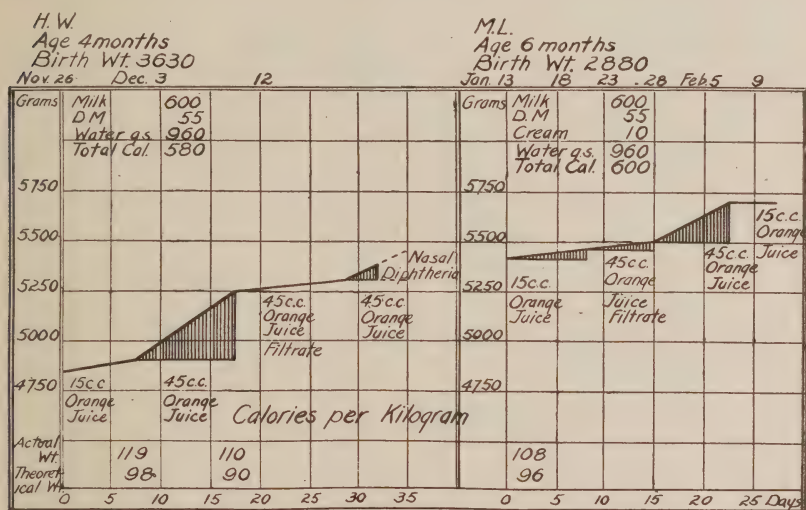


Fig. 3.—Both curves show the comparative influence of the lesser amount of orange juice (15 c.c.), the filtrate of the kaolin treated orange juice (45 c.c.) and of the large amount of untreated orange juice (45 c.c.).

vitamin) grew quite as well as those receiving the untreated orange juice (Fig. 5, Group 2). Furthermore, the addition to a purified ration of the kaolin residue from the orange juice produced a prompt resumption of growth in other animals in which the orange juice filtrate had failed to secure growth (Fig. 5, Group 3, Period 2).

That orange juice contains a considerable quantity of the anti-neuritic vitamin was also shown by its effect on polyneuritic pigeons. These birds, previously fed polished rice for from twenty-one to thirty-seven days, developed typical polyneuritis, manifested by the classical symptoms—muscular weakness, retraction of the neck, and paralysis of the muscles of deglutition. One of these pigeons, suffer-

ing from almost complete paralysis of respiration, was quite restored by the next morning, after the subcutaneous and oral administration of orange juice on the previous evening. The orange juice, which was injected subcutaneously, was made neutral with sodium hydroxid and sterilized.

The difference in the curative effect of the treated and untreated orange juice was strikingly brought out in two polyneuritic pigeons. One bird, receiving daily 10 c.c. of the untreated juice, recovered in twenty-four hours and showed no polyneuritic symptoms thereafter; the other pigeon, which was given each day 10 c.c. of the filtrate from the kaolin treated orange juice died after four days. A fourth polyneuritic pigeon became so weak that when placed on its side it could

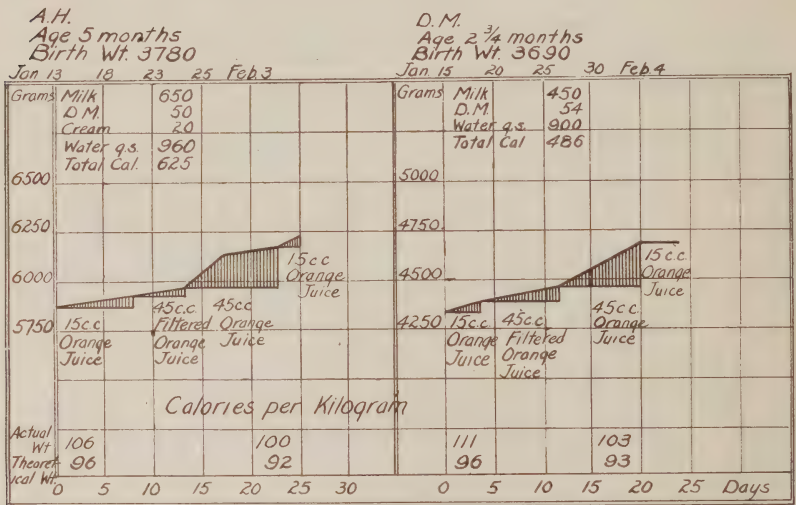


Fig. 4.—The curves also illustrate the difference between the effect of the removal of the antineuritic vitamin from orange juice.

not resume the upright position. It was given 10 c.c. of untreated orange juice by mouth. The next morning the bird was up and about, and apparently quite normal. At the necropsy, the polyneuritic pigeons were found to have full crops and dilated hearts. Some pericardial fluid was present.

To determine the effect of the filtrate from the kaolin treated orange juice, a series of observations were made on both rats and guinea-pigs. Rats (Fig. 5, Group 3, Period 2), fed a purified ration to which this filtrate previously neutralized was added, made no growth, although the amount used was equal to that of the groups fed the untreated and alkalinized orange juice. Guinea-pigs in which scurvy had been produced by a prolonged diet (sixty days) of oats and 40 c.c. of super-

heated milk (100 C. for one hour) per day were cured by the addition of 5 c.c. per day of the filtrate of the kaolin treated orange juice. These facts lead us to conclude that orange juice shaken with kaolin and filtered loses its growth stimulating property, while its antiscorbutic potency is not impaired. Our results, here, are in keeping both with our observations on babies and with the work of Harden and Zilva.

DISCUSSION

The results obtained by the addition of orange juice to or omission from the diet of babies were uniform and constant. Under the conditions maintained, growth, as evidenced by the weight curves, was in all cases stimulated when orange juice was given. On the other hand, orange juice from which the antineuritic vitamin had been removed was without influence. The fact that the changes produced were usually apparent within a day made the results more significant. That

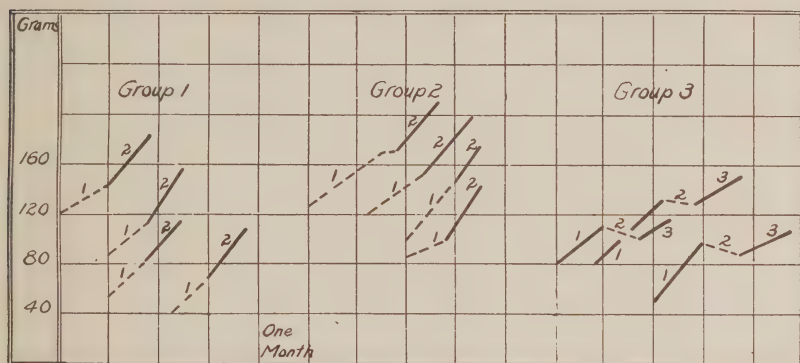


Fig. 5.—This shows the effect on the growth of rats of the addition of the treated and untreated orange juice to a purified ration otherwise complete but lacking the antineuritic vitamin. The ration consisted of 18 per cent. casein, 5 per cent. butter, 23 per cent. lard, cornstarch 46.79 per cent., and 7.03 per cent. of a suitable salt mixture. During Period 1, Group 1, the ration contained 55 c.c. of orange juice per hundred grams of ration; during Period 2, 75 c.c. of orange juice were used. Group 2 was given the alkalinized orange juice, 55 c.c. being added to the purified ration during Period 1, and 75 c.c. during Period 2. During Period 1, Group 3 was given the purified ration in which our wheat embryo extract (25 c.c. per hundred grams) was the sole source of the antineuritic vitamin. In Period 2, 75 c.c. of the filtrate from the kaolin treated orange juice was added to the purified ration. During Period 3, the source of the antineuritic vitamin was the kaolin residue of the shaken orange juice.

other constituents of the orange juice, for example, the carbohydrates, apparently played no part in our results is shown in the case of A. H. L. Dietary increases, both during and after an orange juice period, were without marked effect. Furthermore, the addition of 3 gm. of sugar

— dextri-maltose — an amount equivalent to the sugar content of the orange juice of the previous period was also without appreciable effect (Fig. 1). In certain instances the gains were less marked than in others, the greatest gains being made in those babies receiving the most food based on their theoretical weight. If the caloric intake per kilogram—that is, kilogram of estimated weight—fell to ninety or thereabout, there was less stimulation. This is shown in Figure 4.

Up to the present time studies dealing with the influence of the antineuritic vitamin on growth have not shown whether the weight increases were due to the stimulation of appetite, and thus an increased ingestion of food, or to the direct influence of the antineuritic vitamin on metabolism.¹⁴ While not attempting to solve this problem, we have watched our children with this point in mind. In no case was a loss of appetite apparent, the same amount of food being taken during the entire experimental period. In animal experiments the conditions are quite different; for in these considerably less than the minimal requirement of the antineuritic vitamin is usually given for a much longer time. Under these conditions the appetite is greatly diminished; when the antineuritic vitamin, therefore, is added, the effect on the appetite is marked. In our babies' diet, however, a considerable amount of this essential growth constituent was always present, and, therefore, the appetite factor did not seem to enter into consideration. Furthermore, we recall the paralyzing influence of the polished rice diet on the gastro-intestinal tract of the polyneuritic pigeons and wonder if it is not this phenomenon which in animal experiments, in part, at least, is responsible for the anorexia. In general, it appears that the appetite factor plays only a minor rôle in the stimulating effect of the water soluble vitamin on growth, provided a nearly adequate amount is being given.

The question as to whether the antiscorbutic vitamin has growth stimulating properties has not been the subject of extensive experimentation. All authors agree that there is a loss of weight in experimental scurvy, especially as the manifestations become more distinct. There is, however, a corresponding loss of appetite which may be responsible for this. To be sure, in the many observations made by Hess, a failure to gain in children was usual; and in the early stages of the disease, at least, there was no actual loss in weight. Harden and Zilva,¹⁵ and Drummond¹⁶ have reported that rats fed a purified ration to which a small amount of orange juice was added as an antiscorbutic made better gains than others similarly fed but without the

14. Osborne, T. B., and Mendel: *J. Biol. Chem.* **37**:187 (Jan.) 1919.

15. Harden, A., and Zilva, S. S.: *Biochem. J.* **12**:408 (Dec.) 1918.

16. Drummond, J. C.: *Biochem. J.* **13**:77 (May) 1919.

orange juice. They concluded from their work that the antiscorbutic vitamin was essential to the well-being of animals, as manifested by the better weight gains of the rats receiving it. This suggests that the antiscorbutic vitamin has growth stimulating properties. Our own observations on babies, however, fail to bear this out.

At the present time there is little information regarding the coexistence and quantitative relationship in foods of the two water soluble vitamins—the antineuritic and the antiscorbutic. Orange juice has been demonstrated to contain both in appreciable amounts. Similarly, Hess¹⁷ had found that both are present in the tomato. Other foods which have been found to contain both in demonstrable quantities are banana,¹⁸ cabbage,¹⁹ potato²⁰ and turnip.²¹ It is very probable that this list will be greatly extended by further work.

The pathologic similarities of beriberi and scurvy have been pointed out by a number of workers.²² Funk,²³ however, believed that foods which were specific for scurvy also protected against beriberi, although the “beriberi vitamin” was a prophylactic against beriberi only. At that time he did not appreciate that both vitamins might be present in one and the same food. That certain foods, for example milk, contain both vitamins, may also explain the fact that some of the symptoms of both diseases are sometimes present in one and the same individual, the disease type depending on the greater deficiency of the particular food accessory. Thus, similar heart signs and symptoms are described in both scurvy and beriberi. It is possible that the heart symptoms present in those babies who were suffering from scurvy were due, in part, to a lack of the antineuritic vitamin.

CONCLUSIONS

1. Orange juice contains a relatively large amount of the antineuritic vitamin.
2. The growth stimulating influence of orange juice appears to be due to the antineuritic vitamin contained therein.

17. Hess, A. F., and Unger, L. J.: *J. Biol. Chem.* **38**:293, 1919; *Proc. Soc. Exper. Biol. & Med.* **36**:1, 1918.

18. Sugiura, K., and Benedict, S. R.: *J. Biol. Chem.* **36**:171, 1918. Lewis, H. B.: *J. Biol. Chem.* **11**:91, 1919.

19. McCollum, E. J., and Kennedy, C.: *J. Biol. Chem.* **24**:492, 1916. Cohen, B., and Mendel, L. B.: *J. Biol. Chem.* **35**:425, 1918.

20. McCollum, E. L., and Kennedy, C.: *Loc. cit.*

21. Osborne, T. B., and Mendel, L. B.: *J. Biol. Chem.* **39**:29, 1919.

22. Chicke, H., and Rhodes, M.: *Lancet* **2**:774 (Dec. 7) 1918. Darling, S. T.: *J. A. M. A.* **63**:290 (Oct. 10) 1914.

23. Funk, Casimir: *Ergebn. d. Physiol.* **13**:124, 1913.

3. Orange juice from which the antineuritic vitamin is removed by adsorption does not stimulate growth. This would seem to indicate that the antiscorbutic vitamin lacks growth stimulating properties.

4. The "pathological affinities" of beriberi and of scurvy may possibly be explained by the fact that the antineuritic content of the commonly used antiscorbutics has not been considered.²⁴

24. After this manuscript had been submitted for publication, we found the preliminary note of Mendel and Osborne (Proc. Soc. Exper. Biol. & Med. **17**:46 [Nov. 19] 1919) stating that they were studying the antineuritic content of fruits and had also observed that orange juice contained this growth stimulating material.

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AMERICAN MEDICAL ASSOCIATION
FIVE HUNDRED AND THIRTY-FIVE NORTH DEARBORN STREET
CHICAGO

A DEFICIENCY IN HEAT-TREATED MILKS.

By AMY L. DANIELS AND ROSEMARY LOUGHLIN.

(From the Department of Nutrition, Child Welfare Research Station, University of Iowa, Iowa City.)

(Received for publication, August 9, 1920.)

From time to time during the past few years there have appeared in the literature reports of investigations dealing with the biological value of heat-treated milk. These are more or less contradictory, some authors contending that while certain chemical changes have resulted from the processes employed, they have not altered the biological value of the milk.¹ Indeed, in certain instances it has been pointed out that boiled milk of another species is better borne than raw milk. On the other hand, there are experiments which indicate that the nutritive value of raw milk is much greater than that of boiled milk. These varying results may be due (1) to the different methods employed in heating the milk; or (2) to the different lengths of time the experiments were run. Milk boiled quickly may have quite different values from that which has been brought slowly to the boiling temperature; and an experiment continued over many months may produce results which are not discernible in a short period investigation. Rickets is not a quickly developing syndrome, but the outcome of a greater or less deficiency over a considerable period.

Among those who contend that milk has been made less valuable by heating to high temperatures, there is little unanimity of opinion regarding the cause of the deterioration. Recent findings relative to the thermostability of the antiscorbutic vitamine may account for some of the apparent inconsistencies;² but, in those

¹ The literature has been reviewed by Lane-Claypon, J. E., *Milk and its hygienic relations*, London, New York, Bombay, and Calcutta, 1916, 225.

² Chick, H., Hume, M. E., and Skelton, R. F., *Biochem. J.*, 1918, xii, 131. Hess, A. F., and Fish, M., *Am. J. Dis. Child.*, 1914, viii, 385. Hess, A. F., *Am. J. Dis. Child.*, 1916, xii, 152.

cases where the investigations were carried out with rats, it would seem that the biologic impotency cannot be attributed altogether to the destruction of this vitamine, for rats in our laboratory which have been fed purified rations, containing no demonstrable amount of the antiscorbutic vitamine, throughout a complete life cycle have made what is considered normal growth; have reproduced; and reared their young. These, in turn, have repeated the performance of the parents.

Other substances which have been considered as possibly responsible for the lack of physiologic well being of animals fed heat-treated milk are the casein, and the antineuritic vitamine (water-soluble B). McCollum and Davis³ fed rations in which the sole source of the water-soluble vitamine was superheated whey (15 pounds pressure for 1 hour) in certain cases; and in others the water-alcohol extract of wheat embryo similarly treated. There was no evidence that the vitamine was destroyed. The rats grew quite as well as control animals receiving similar rations containing the unheated materials. When, however, skim milk powder, heated for a considerable period in a double boiler, or for 1 hour in an autoclave at 15 pounds pressure, was used, growth was not comparable to that on the unheated powder. This heated milk powder also lost its potency as a supplementing material for rations consisting of polished rice, salts, and butter fat—rations which require both protein and the water-soluble food accessory to make them support growth. The addition of a growth minimum (10 per cent) of unheated casein to a ration consisting of superheated milk powder stimulated growth, which continued slowly throughout the experiment. The authors conclude that heating casein for 1 hour in an autoclave at 15 pounds pressure quite destroys its biologic value as a complete protein. ●

Hogan,⁴ on the other hand, believes that high temperatures affect the vitamins rather than the proteins. He found that rations including superheated casein and egg white, as essential parts of the protein requirement, produced growth similar to that secured by rations containing the unheated proteins. In a second group of experiments, when corn mixtures, which fur-

³ McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxiii, 247.

⁴ Hogan, A. G., *J. Biol. Chem.*, 1917, xxx, 115.

nished the vitamins, were superheated before the addition of these proteins, growth was less marked than on similar rations which were unheated. Protein deterioration could not have been responsible for the lack of growth here. The vitamins appear to be at fault. There is, however, no experimental evidence to indicate which of the vitamins may have been affected.

Gibson and Concepción⁵ fed fowls a diet of polished rice and milk, some being given raw milk (100 cc.), while others received an equal amount of rice and autoclaved milk. The results indicate that milk has little protective action against polyneuritis, for the birds receiving these additional milks developed polyneuritis in about the same time as those fed polished rice alone. The autoclaved milk, however, did not appear to promote the onset of the neuritic symptoms. When a considerably larger amount (200 cc.) of milk, either fresh or autoclaved, was fed, neither group developed neuritis and there was no evidence of degenerative changes in the peripheral nerves of these birds. From these results it would seem that the antineuritic vitamin had not been materially affected by the high temperatures (2 hours at 125°C.). Comparable results were also obtained with pigs and dogs.

Recently, some suspicion has been cast on the thermostability of the fat-soluble vitamin in certain fats. Osborne and Mendel⁶ allowed steam to pass through butter oil for 2½ hours. This, when used as the source of fat-soluble A in rations, gave every indication of being as efficient as the untreated butter oil. Steenbock and coworkers,⁷ however, have reported that the fat-soluble vitamin is readily destroyed even below 100°C. It is probable that the apparent inconsistency in the results of these investigations lies in the fact that in one case the fat was heated in the presence of water (steam), whereas in the other no moisture was included. We have found no reports dealing with the effects of superheat on the fat-soluble complex as it exists in milk.

⁵ Gibson, R. B., and Concepción, I., *Philippine J. Sc., Section B*, 1916, xi, 119.

⁶ Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1916, xxiv, 37.

⁷ Steenbock, H., Boutwell, P. W., and Kent, H. E., *J. Biol. Chem.*, 1918, xxxv, 517.

In a recent study (1916) of the nutritive value of milk held at the boiling temperature for different periods, Daniels and Stuessy⁸ observed that rats fed milk boiled 1, 10, and 45 minutes, respectively, grew slowly, failed to achieve the expected weight for normal animals, and never reproduced. Growth curves of rats receiving milk heated above 100°C. (114°C. for 45 minutes) were fairly comparable to those receiving milk held at the boiling temperature, nutrition disaster intervening only somewhat earlier. The addition of well washed coagulated egg yolk and casein to the diets of those animals receiving the milk held at the boiling temperature for 45 minutes, and of coagulated egg white to the superheated milk, caused growth to be resumed. The animals fed the egg yolk additions produced several litters of young which were successfully suckled. Although the authors offer in explanation of their results the possible destruction of some of the casein, which was supplemented by the added protein, they suggest that there might be other contributing factors, such as the vitamins and inorganic constituents which were responsible for the growth stimulation.

Since the first report, some of the above experiments have been repeated with the view of determining, if possible, what substance or substances are changed by the heating processes. In the earlier work, in order that all conditions during heating might be as nearly comparable as possible, equal quantities of milk (1 pint) were brought to the desired temperature in glass containers of the same size and form, which were completely surrounded by cold water. The period of boiling was counted from the time the milk reached the boiling temperature (98°F.), the time required to bring the milk to the boiling point (about 35 minutes) not being considered. In the new experiments, since the length of time the milk was held at the boiling temperature appeared to make but little difference in the growth of the animals, the milk was brought quickly to the boiling point in an open aluminum kettle, and the boiling continued for just 1 minute. At that time it was believed that milk brought quickly to the boiling point was in every way comparable to that heated by the former method. In these experiments special care was taken

⁸ Daniels, A. L., and Stuessy, S., *Am. J. Dis. Child.*, 1916, xi, 45.

to select well nourished young rats from our stock group. Our rats of the first generation fed this quickly heated milk grew normally, and to all appearances were perfectly nourished animals (Chart 1). Growth in these rats was in such striking contrast to that of the earlier work that it seemed best to repeat the former experiments, heating the milk under the same conditions as before. Our results with this second group of rats fed the milk brought slowly to the boiling point were the same as those

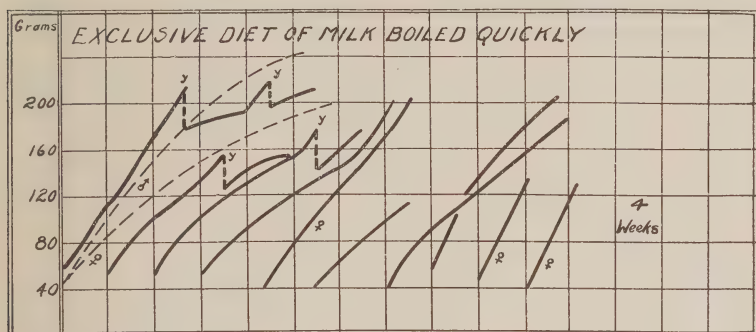


CHART 1. These animals were fed milk heated *quickly* to the boiling temperature and sustained for 1 minute.

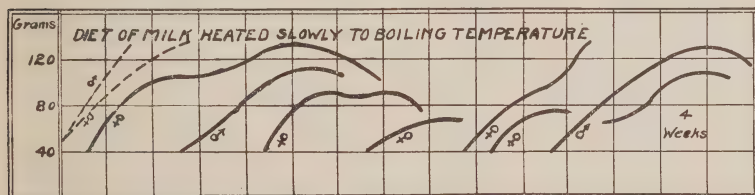


CHART 2. Rats fed milk heated slowly to the boiling temperature grew at about half the usual rate.

of the first experiments—the animals grew slowly, reached only about half the normal size, and failed to reproduce (Chart 2). A comparison of the growth curves of these milk-fed animals can lead to but one conclusion; namely, that milk brought quickly to the boiling temperature and held there for only a short period is little affected, while that which has been heated for a considerable period (35 minutes), even below the boiling temperature, is so changed that it fails to meet the nutritive requirements of rats.

The results with the slowly heated milk led us to investigate the effects of commercially canned milk and milk pasteurized by the "hold" system—a system used extensively in dairies and in institutions where numbers of babies are cared for. Previous experience in our laboratory with milk pasteurized by the "flash" system has indicated that it is little affected by the process. Rats grew as well on this as on milk raised quickly to the boiling temperature.

The pasteurized milk used in our experiments was prepared under the same conditions as that used for the babies in our children's hospital. The milk was placed in individual nursing bottles which were surrounded by cold water. The water was heated by steam to 65°C. in some cases, and in others to 82°C., the time required for heating varying from 30 to 45 minutes, depending upon the pressure on the particular day. The milk was then held at the desired temperature 40 minutes, after which it was cooled by running water and placed on ice.⁹

In our experiments with commercially canned milk, both the sweetened (condensed) and the unsweetened (evaporated) were used. In the latter case (evaporated milk) three brands, designated as Brands A, B, and C, were tested. In one case only were we able to get definite information regarding the methods of preparation. We infer that the methods employed in the other two cases were similar. The process consists in holding the milk at the boiling temperature for approximately 10 minutes. It is then evaporated *in vacuo* at a temperature of 130–140° F. until the ratio is approximately two to one. After cooling, it is canned, sealed, and sterilized at 240° F. for about 20 minutes. These milks diluted with equal quantities of distilled water, to which a few specks of iron citrate were added, were fed *ad libitum*, especial care being taken to see that an abundance of milk was always provided.

In the production of the condensed milk tested according to the statement of the manufacturer, after adding the sugar the

⁹ We appreciate that the temperature of pasteurization was somewhat higher than that used in the commercial process, and therefore no conclusions regarding the biologic value of the commercially pasteurized milk can be drawn. Experiments with milk commercially pasteurized are now in progress.

milk is first heated to approximately 200° F. This is then condensed to the desired consistency in vacuum pans, the temperature during this part of the operation averaging 150° F., but as the milk condenses the temperature gradually drops until it reaches about 120° at the completion of the process. This milk was fed undiluted, distilled water being provided in other containers.

The growth curves of the rats fed the pasteurized milk (Chart 3) are very similar to those of animals which received the milk heated slowly to the boiling temperature. As in the latter case,

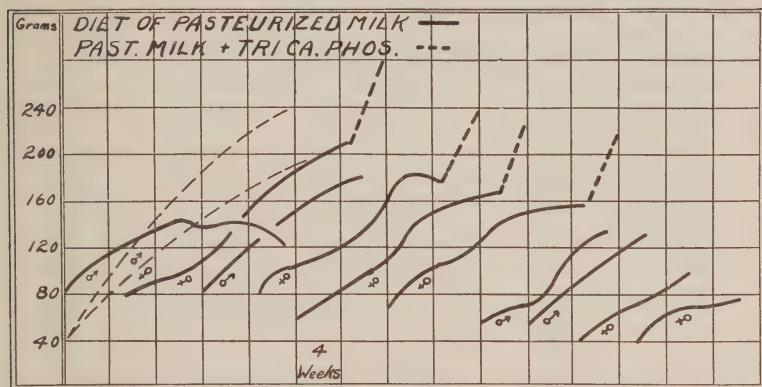


CHART 3. Growth curves of rats fed milk pasteurized by the "hold" process in the apparatus used for pasteurizing the hospital infants' milk feedings. During Period 2 (represented by the broken line) the addition of tricalcium phosphate incorporated in a starch paste stimulated growth.

the rats grew at about half the usual rate and never attained the normal size for adult animals.

With the unsweetened (evaporated) milk the results were even more surprising (Chart 4). On Brand A the animals made almost no growth gains and died after a few weeks. On Brands B and C slightly better results were obtained. The animals gained slowly and lived for somewhat longer periods, but all ultimately died in a miserable condition—emaciated, with roughened coats, but with no signs of xerophthalmia. The somewhat better results with Brands B and C may possibly be explained by the fact that the animals on these were about 2 weeks older when the experiment was begun.

The animals fed the sweetened (condensed) milk¹⁰ made decidedly better growth than any of our rats fed the other forms of long heat-treated milk; in fact their curves of growth are fairly comparable to those of normal animals (Chart 5, Group 2). Although the temperatures used in the process of condensing,

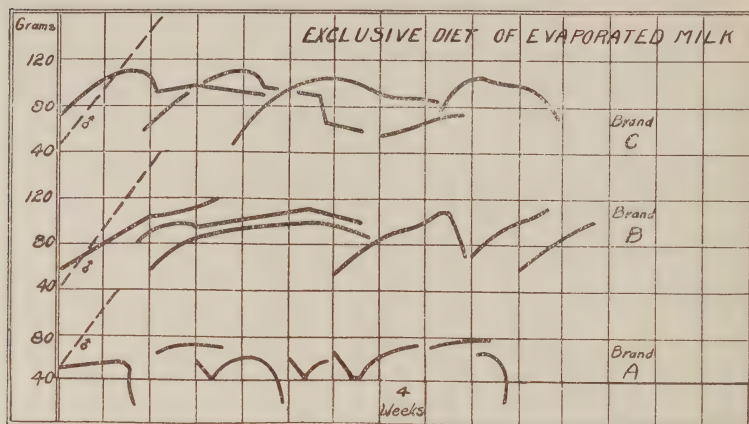


CHART 4. Animals fed evaporated milk (unsweetened) made scarcely no growth and died after a few weeks.

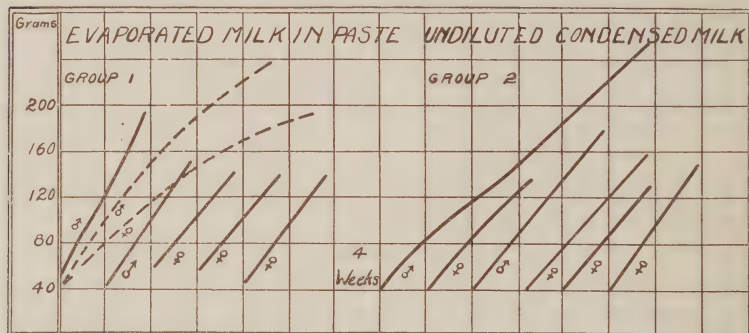


CHART 5. Group 1 indicates the rate of growth of animals fed evaporated milk made into a starch paste.

Group 2 are growth curves of animals fed undiluted condensed (sweetened) milk.

¹⁰ We are indebted to Miss Emma Francis, Battle Creek, Michigan, for assistance in these experiments.

after the initial step, were not so very different from those employed in our pasteurization processes, the chemical changes produced, seemingly, had not taken place. In view of our other findings these results were at first confusing. The explanation, however, was forthcoming later in the investigation.

In studying what changes had taken place in the unsweetened (evaporated) and our long heat-treated milks, our attention was first directed toward the possible destruction of the two vitamins, fat-soluble A and water-soluble B. The addition of liberal amounts (25 cc. per 200 cc. of milk) of the water-alcohol extract of wheat embryo to the milk raised slowly to the boiling temperature produced no perceptible change in the growth curves of these animals. Nor was there any evidence that the antineuritic vitamin was affected in the milk heated to the higher temperatures (evaporated milk). Those animals fed purified rations¹¹ in which the antineuritic material was supplied by fresh milk in one case and diluted evaporated milk in another, gave no indication that the antineuritic vitamin was destroyed by the condensing process (Chart 6). During the early part of the investigation too little milk (less than 200 cc. per 100 gm. of ration) was added to produce normal growth. The growth curves of the animals receiving the evaporated milk additions, however, were consistently better than those of animals receiving the fresh milk mixture. These better results seem to point to a higher antineuritic content of the food mixture containing the evaporated milk. In preparing the foods especial care was taken to dilute the evaporated milk with equal quantities of distilled water. A comparison of the protein ($N \times 6.25$) content of this diluted milk (3.5 per cent) and the fresh (3.3 per cent) milk suggested that the evaporated milk, as fed, might contain slightly more of the vitamin. We appreciate, however, that the amount of protein

¹¹ The purified rations consisted of:

Casein.....	13.5 gm.
Corn-starch.....	77.0 "
Suitable salt mixtures.....	6.0 "
Milk, evaporated.....	28.0 cc.
" fresh.....	56.0 "

The precipitated casein was washed 24 hours in running water, dissolved in 0.2 per cent sodium hydroxide solution, reprecipitated with diluted acetic acid, and again washed 24 hours in running water.

a milk contains can be, at best, only a very rough estimate of its antineuritic value. Nevertheless, if the antineuritic vitamine had been considerably destroyed by the prolonged heat treatment employed in the evaporation processes, it would have been evidenced, we believe, by a slower growth rate of the animals fed the evaporated milk ration.

We have been unable also to obtain data indicating that the fat-soluble vitamine is appreciably affected, if at all, by the heat treatment of milk. The addition of butter oil (2 gm. per 100 cc. of diluted milk) was without effect in stimulating growth

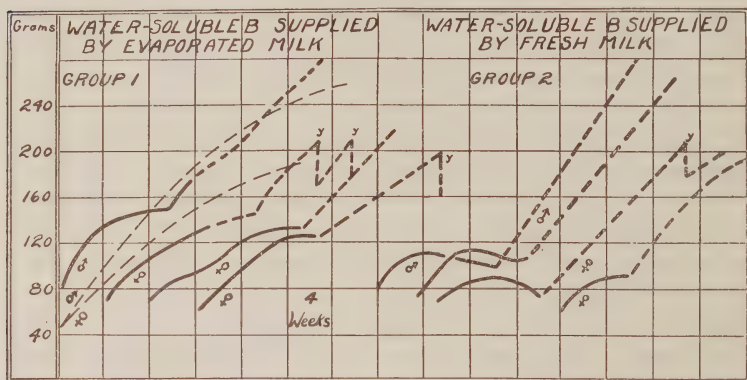


CHART 6. Animals fed purified rations in which evaporated milk (100 cc. during Period 2), Group 1, and fresh milk (200 cc. during Period 2), Group 2, respectively, supplied the only water-soluble vitamine made comparable growth gains.

in the stunted animals fed the superheated (evaporated) milk. A comparison of the growth curves of animals fed a purified ration in which the fat-soluble vitamine was supplied very largely by 2 per cent of milk fat from evaporated milk (28 cc. per 100 gm. of ration), and fresh milk (56 cc. per 100 gm. of ration), respectively, gave no evidence of the destruction of this vitamine (Chart 7). During the period of investigation both groups have made normal growth gains, and in neither has there been any indication of xerophthalmia. The purified ration for these particular groups was prepared from casein obtained from centrifuged milk. This was not ether-extracted, nor was the wheat embryo used as

the source of the water-soluble vitamine extracted previous to the alcoholic treatment. It is obvious, therefore, that somewhat more than 2 per cent of butter fat was being fed. But since it has been shown that 5 per cent is essential for normal growth,¹² it was believed that if a considerable destruction of the butter fat had taken place in the superheated milk it would be made apparent by the comparative study.

The addition of both vitamins—the water-soluble and the fat-soluble—produced no growth stimulation in the stunted

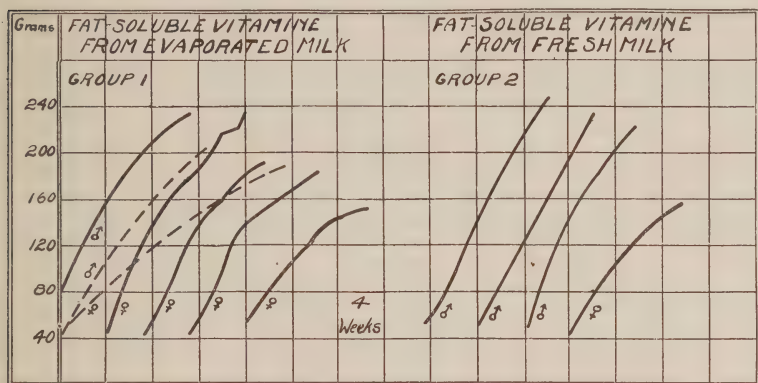


CHART 7. Growth curves of animals fed purified rations in which the fat-soluble vitamine was supplied by 2 per cent of fat from evaporated milk (Group 1), and fresh milk (Group 2). In these rations the casein and wheat embryo which furnished the extract containing the water-soluble vitamine were not ether-extracted.

animals fed the evaporated milk. If either of these vitamins was affected by the methods of heating, it was not indicated by our investigation.

The insoluble precipitate on the sides and in the bottom of some of the cans of evaporated milk used in the investigation suggested that the inorganic constituents, in part at least, might be responsible for our growth failures. It is well known that boiling brings about changed relations in the inorganic complexes of the milk, resulting in an increase of the insoluble calcium and

¹² McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xx, 641.

magnesium salts, especially the calcium phosphates,¹³ at the expense of the soluble forms. But all evidence in stock feeding, at least, points to the conclusion that these di- and tri-basic salts are available. Bone ash and precipitated calcium phosphate are usual additions to certain types of farm rations.¹⁴ The additions of mono-basic and di-basic calcium phosphate, respectively, to our milk foods were without significant influence. The mono-basic salt was apparently distasteful, for when this was added very little of the milk was eaten. With the di-basic phosphate somewhat better results were obtained, but growth was still considerably below normal. When calcium lactate was

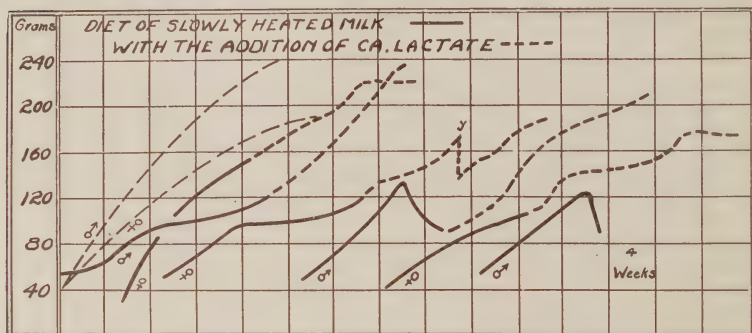


CHART 8. The addition of calcium lactate to milk brought slowly (35 minutes) to the boiling point, stimulated growth (Period 2). The rate of growth, however, is considerably less than the optimum.

added to the milk brought slowly to the boiling point, growth was stimulated in animals previously stunted on this milk alone (Chart 8). Furthermore, young animals fed both the long heat-treated milk, and the superheated milk (evaporated) to which calcium lactate (approximately 0.77 gm. per 100 cc. of milk) was added from the beginning, made fairly satisfactory growth gains; and in a number of cases young were born (Charts 9 and 10). Very occasionally we succeeded in raising a few of a second generation. However, the number of young which we failed to

¹³ *Bull. Hyg. Lab., U. S. P. H. No. 56*, 1909, 2nd edition, 646.

¹⁴ Henry, W. A., and Morrison, F. B., *Feeds and feeding*, Madison, 16th edition, 1916, 66.

raise, and the fact that our first generation was somewhat below the accepted standard for well nourished rats, suggested that calcium lactate only in part made up the deficiency produced by the heat treatment of milk. The additions of considerably larger amounts of calcium lactate produced no better results.

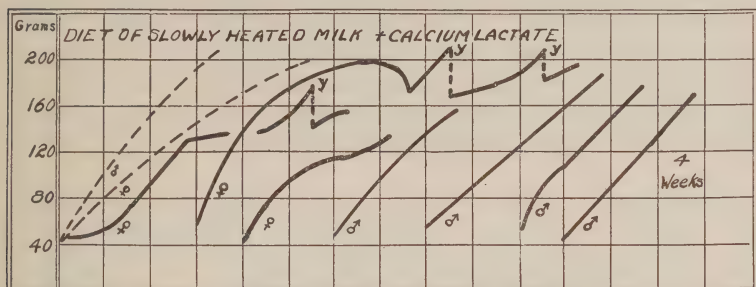


CHART 9. These animals were fed, from the beginning of the experimental period, milk brought slowly to the boiling temperature, to which calcium lactate was added.

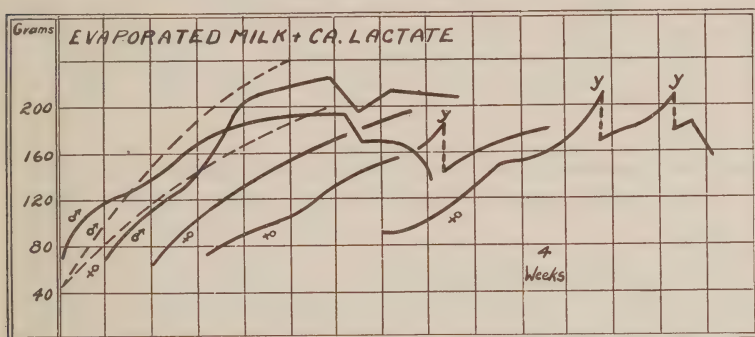


CHART 10. The addition of calcium lactate to evaporated milk produced much better growth than evaporated milk alone. In some cases young were born; a few lived through the suckling period.

It seemed probable that our lack of success with the dicalcium phosphate might have been due to the fact that enough of the salt could not be dissolved in the milk to meet the needs of the experimental animals, therefore the more soluble calcium glycerophosphate was tested. The substitution of this for the calcium

lactate stimulated growth (Chart 11, Group 1). By incorporating the calcium glycerophosphate in a paste of diluted evaporated milk and corn-starch, our results were even more successful. The growth curves of these animals (Chart 11, Group 2) were quite similar to those of animals fed milk boiled 1 minute. With our calcium glycerophosphate additions young rats have grown to maturity and reproduced at a comparatively early age. This is the first time in our many experiments with long heat-treated, or superheated milk that we have been able to secure results at all comparable with those obtained on raw milk (Chart 12) or milk brought quickly to the boiling point (Chart 1).

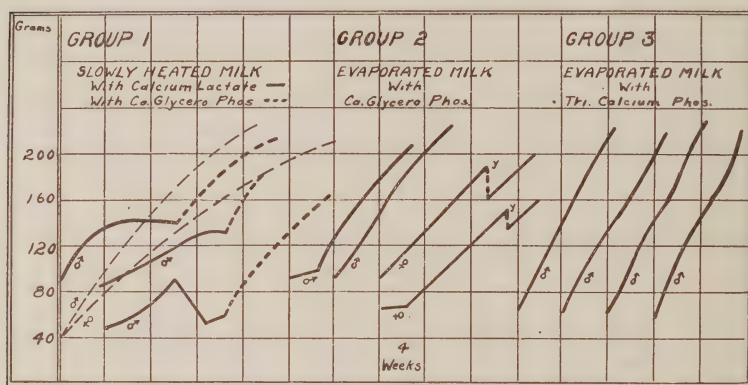


CHART 11. The substitution of calcium glycerophosphate for calcium lactate in the diet of rats fed slowly heated milk stimulated growth (Group 1).

Calcium glycerophosphate added to evaporated milk produced normal growth curves. Young were born at a comparatively early age (Group 2).

Evaporated milk supplemented with tricalcium phosphate in a starch paste produced equally good growth (Group 3).

As has been stated, all evidence in the literature is to the effect that tricalcium phosphate when fed to stock is available. Do rats differ from farm animals in being unable to utilize this tri-basic salt? In order to determine this we fed evaporated milk (Brand A), and supplemented this with the insoluble salt incorporated in a starch paste. The growth of these animals (Chart 11, Group 3) is superior to that of any of our milk-fed rats,

and leaves no doubt concerning the availability of this tri-basic salt.

Why then did our animals fail to grow on the long heat-treated and evaporated milk? Two possibilities suggest themselves. In the process of condensing, a certain amount of the calcium phosphate is precipitated and may be discarded as waste, although in the published analyses¹⁵ there is no evidence that such has been the case. It is also possible that some of the insoluble material settled to the bottom of the feeding containers and was not taken by the animals. Rats fed the diluted evaporated milk (Brand A) thickened with corn-starch made practically normal

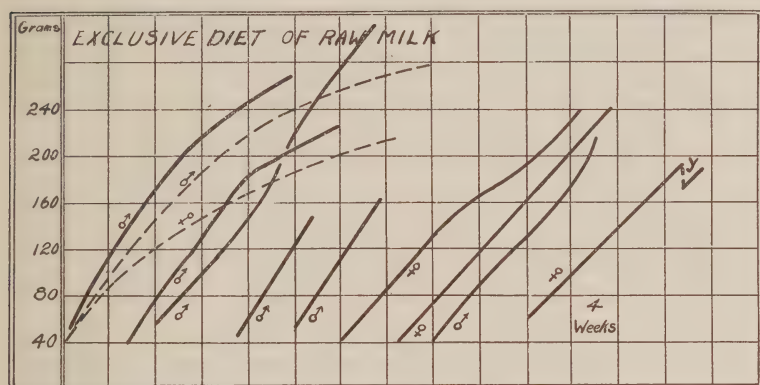


CHART 12. Growth curves of first generation rats fed raw milk.

growth gains during the 8 weeks of the investigation (Chart 5, Group 1). A comparison of these curves with those of our animals on the unthickened evaporated milk suggests that the growth failures of our animals on the evaporated milk were due to the fact that the insoluble calcium phosphate in the diluted milk settled out and was not eaten.

Can the nutritive failures of our animals fed the long treated milk, that is the milk brought slowly to the boiling point, and the pasteurized milk, be similarly accounted for? In these we have never been conscious of any considerable precipitate.

¹⁵ Sherman, H. C., Chemistry of food and nutrition, New York, 2nd edition, 1918, 424.

although slight precipitates were frequently observed on the sides and bottom of the containers in which the milks were heated. In order to test this point, animals previously stunted as the results of pasteurized milk feeding were given in addition the tricalcium phosphate starch paste. As in the case of the evaporated milk-fed rats growth was immediately stimulated (Chart 3), suggesting that in the "hold" process of pasteurization the insolubility of the calcium salts was responsible for the growth failures. When the bottles in which the milk was pasteurized were carefully washed out with distilled water and the washings incorporated in a starch paste, the animals made better gains

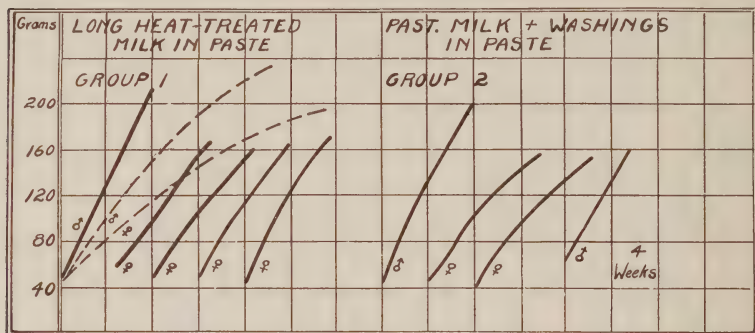


CHART 13. Long heat-treated milk made into a paste also produced growth superior to that of the long heat-treated milk alone (Group 1).

The washings from the containers in which the milk was pasteurized, when added to the pasteurized milk in the form of a paste, produced better growth than the pasteurized milk alone (Group 2).

than on the pasteurized milk alone (Chart 13, Group 2). Similarly, our animals fed long heat-treated milk incorporated into a paste made normal growth gains (Chart 13, Group 1). In both these cases the results can be explained only by the fact that the calcium phosphates were held in suspension by the colloidal solution and therefore were made available.

The growth of the animals fed the thickened heat-treated milk suggested an explanation for our results with the condensed milk. This milk, it will be recalled, is of a thick, semisolid consistency, and in our experiments it happened that this was fed

undiluted. Any insoluble calcium salts formed during the condensing process, therefore, were held in suspension. The thick mixture made it possible for the animals to get all the calcium phosphates present.

The explanation for the results obtained in the previous investigation⁸ following the addition of egg yolk and casein to the long heat-treated milk, is now apparent. Both of these materials furnished appreciable amounts of calcium and phosphorus—substances which have been shown to be deficient in long heat-treated milk.

The results of all our experiments on the long heat-treated milks point to the same conclusion; namely, that, in the process of heating, the calcium salts are rendered more or less insoluble, depending upon the length of time the milk is heated. In this insoluble form they may be lost, owing to the fact that some of the precipitated material adheres to the container, as in the case of long pasteurized or slowly heated milk, while some, for example in evaporated milk, separates out on standing. When especial care was taken to include the insoluble material by colloidal suspension, results comparable to those on raw and quickly boiled milk were obtained. We have secured no data indicating that either the fat-soluble or the water-soluble vitamine in milk is affected by heat treatment. Nor is the casein apparently affected. Rats fed superheated milk supplemented with calcium phosphate properly incorporated made normal growth gains. The inferior growth of rats on the long heat-treated and superheated milk appears to be due wholly to the readjustments of the inorganic complexes.

The application of these findings to infant nutrition is now under investigation.

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